IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Oreste, *et al.* Group Art Unit: 1623
Serial No.: 10/582,687 Examiner: Layla Bland
Filed: June 13, 2006
Title: LOW MOLECULAR WEIGHT POLYSACCHARIDES HAVING ANTITHROMBOTIC ACTIVITY

Commissioner for Patents
P.O. Box 1450
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DECLARATION PURSUANT TO 37 C.F.R. §1.132

Sir:

I, Pasqua ORESTE, biochemist graduated in Biology at the University of Milan (Italy) do hereby declare as follows:

1. I am joint inventor of instant application and jointly invented the whole subject matter claimed therein with Giorgio Zoppetti. I am familiar the instant application and have read the official action mailed on November 26, 2007.
2. I am also familiar with literatures regarding polysaccharide K5 and its semisynthetic derivatives, in particular with the cited publications Naggi et al., Carbohydrate Chemistry, 2001, 336(4) 283-290, which I am a co-Author of, and WO 02/50125, which I am a designated co-inventor of.
3. Under my supervision, the biological parameters for the prediction of the antithrombotic activity, i.e. the Anti-Xa, Anti-IIa (antithrombin) and APTT (Activated Partial Thromboplastin Time) activities of
   - the product of Example 1 of instant application, as test compound;
   - commercial unfractionated heparin (UFH), 5000 IU/ml with specific Anti-Xa and anti-IIa activities of 160 U/mg and 160 U/mg, respectively (data from manufacturer); and
   - commercial dalteparin as a low molecular weight heparin (LMWH) with specific Anti-Xa and anti-IIa activities of 155 U/mg and 60 U/mg, respectively (data from manufacturer), as reference compounds,

   were evaluated in August 2003.

   Standard: International "Low-Molecular-Mass Heparin" standard, as supplied by European Pharmacopoeia Commission, batch 2, lot 2e, with specific Anti-Xa and Anti-IIa activities 106.1 U/mg and 44.2 U/mg, respectively.
4. The employed evaluation method was that described in detail in paragraphs [0121]-[0124] of instant application published as US 2007/0155694 A1. The obtained results are summarized in Table 1, wherein
   - column 1 lists the testes compounds;

- column 2 shows the Anti-Xa activity in International Units per mg (IU)/mg;
- column 3 shows the Anti-IIa activity in IU/mg;
- column 4 shows the ratio of the Anti-Xa/Anti-IIa activities ratio;
- columns 4 and 5 show the dose causing a coagulation time of 100 sec and the APTT activity, in IU/mg) calculated therefrom, respectively.

Table 1

Test Compound	Anti-Xa IU/mg	Anti-IIa IU/mg	Anti-Xa/ Anti-IIa	APTT activity	
				$EC_{100\ s\ in\ APTT}$ (in µg/ml)	Activity (in IU/mg)
Example 1	118	43	2.7	25.5	43
UFH	272	162	1.7	5.2	n.c.
LMWH	217	85	2.6	3.4	n.c.

n.c. = not calculated

Table 1 shows that the anti-Xa activity of UHF is higher than that given by the manufacturer. This is due to the fact that sLMWH has been used as standard. The other activities appeared as expected. The Anti-Xa and Anti-IIa activities of the compound of Example 1 were approximately 50% of those of LMWH heparin and, by consequence, the anti-Xa/Anti-IIa ratio was also similar. In increasing coagulation time, the compound of Example 1 was clearly weaker than the reference compounds. In comparison to UFH and LMWH, approximately 5 to 8 fold doses of compound of Example 1 were needed to produce the coagulation time of 100 seconds.

5. The data given in item 4 above are so markedly different from those given in Example 2 of the cited reference WO 02/50125 that, scientifically, a direct comparison in a specific experiment is unnecessary in order to demonstrate that the involved products have a completely different biochemical profile as far as the essential parameters for predicting an antithrombotic activity are concerned.

   The sulfation degree and sulfate group distributions of the LMW product of Example 2 of Oreste et al. WO 02/50125 and of that of Example 1 of instant application are collected in Table 2, said data being taken from the values given in said examples.

Table 2

Degree of Sulphation and Sulfate Groups Distribution	*Ex. 2 c.r.**	*Example 1*
Sulfate to carboxyl ratio	2.55	2.8
N-sulfate content	>90%	>90%
6-O sulfate content	85%	80%
3-O sulfate glucosamine content	20%	50%
Iduronic acid 2-O sulfate content	25%	20%
Glucuronic acid 3-O sulfate content	30%	40%
Unsulfated uronic units content	40%	40%
Iduronic acid 3-O sulfate + glucuronic 2-O sulfate content	5%	0

(*) Cited reference

The main difference between the two products resides in the higher percent of O-sulfate groups in the position 3 of glucosamine and in the absence of concurrent iduronic acid 3-sulfate and glucuronic acid 2-sulfate groups, notwithstanding the higher sulfation degree of the product of Example 1 of instant application in respect of that of Example 2 of WO 02/50125.

The anti-Xa, Anti-IIa and APTT activities given in item 4 above for the product of Example 1 of instant application and the same activities given for the product of Example 2 in WO 02/50125 are summarized in Table 3. The activity data of the product of Example 2 of WO 02/50125, which are expressed as the percent of activity against the International Standard of Heparin in said document, have been converted into International Units by milligram (IU/mg)

Table 3

Coagulation Parameters	***Example 2, c.r.***		***Example 1***	
	Activity	**%***	**Activity**	**%***
Anti-Xa (UI/mg)	158 IU/mg	99%	118 IU /mg	73.7%
Anti-IIa (UI/mg)	325 IU/mg	203%	43 IU /mg	26.9%
APTT	83 IU/mg	52%	32.6 IU /mg	20.4%
Anti-Xa/Anti-IIa ratio	0.49		2.7	
Anti-Xa/APTT ratio	1.9		3.6	

* in respect of Heparin Fourth International Standard having a value of 160 IU/mg for the three parameters

Table 3 shows that the product of Example 2 of WO 02/50125 is a powerful antithrombin (Anti-IIa) agent, as it is stated in the title and throughout the specification of WO 02/50125, while the product of Example 1 of instant application has a lessened Anti-IIa activity (i.e. it is a low antihrombin agent). In addition, the APTT activity of the product of Example 2 of WO 02/50125 is more than 2.5-fold higher than that of Example 1 of instant application, thus predicting a lower hemorrhagic risk involved by the use of the product of Example 1.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the making of willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the applications or any patent issuing thereon.

Respectfully submitted,

Dated: March 3, 2008

Pasqua ORESTE